REMARKS

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 2, 4, 13, 15-16, 20, and 23 are amended, claims 1, 5 and 7 are canceled, and claims 54-57 are added; as a result, claims 2, 4, 6, and 8-57 are pending.

Claim 2 was objected to because of an informality. The amendment to claim 2, to insert "increased", addresses the objection thereto.

The 35 U.S.C. § 112 Rejections

Claims 1-2, 4-7, 13, 15-16, 20, and 23 were rejected under 35 U.S.C. § 112, first paragraph, as lacking adequate description. Claims 1-2, 4-7, 13, 15-16, 20, and 23 were also rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. These rejections, as they may be maintained with respect to the pending claims, are respectfully traversed.

The Examiner asserts that in analyzing whether the written description requirement is met for agent genus claims, it is first determined whether a representative number of species have been described by their complete structures, and next it is determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics, specific features and functional attributes that would distinguish different members of the claimed genus. The Examiner asserts that in the instant case, doxorubicin is the only species whose complete structure is disclosed to possess the functional property of inhibiting the expression of amiloride-sensitive epithelial sodium channels (ENaC) having α, β and γ subunits of ENaC, and that agents with the other identifying characteristic (for example, inhibition of transcription of one or more ENaC subunit genes, alteration of the level, amount or activity of a molecule that alters ENaC transcription, alteration of ENaC RNA stability, and/or alteration of the trafficking and processing of molecules, for instance, molecules of non-viral origin through intracellular compartments, including without limitation proteosomes, endosomes, and trans-Golgi, and/or through the cytosol, e.g., via cytoskeletal components such as microtubules or microfilaments), vary greatly in structure and function. The Examiner continues, asserting that

the specification does not disclose how an artisan would have identified and differentiated one structurally and functionally distinct compound that possesses any one of such properties from another compound that might possess such properties.

The claims are directed to a method of screening agents having a particular activitiy for a specific second activity. It is Applicant's position that agents that enhance viral transduction and methods to identify those agents are well known to the art (see, for example, Duan et al., J. Clin. Invest., 105:1573 (2000), a reference cited against the claims under § 103, and Examples 1-2, 4-5 and 7 in the specification; and the abstracts for Ravot et al., J. Gene Med., 4:161 (2002); Rieger and Kipps, Cancer Res., 63:4128 (2003); Themis et al., Gene Thera., 5:1180 (1998); Cheng et al., Antimicro. Agents Chemother., 48:2437 (2004) and Dhanak et al., J. Biol. Chem., 277:38344 (2002); a copy of each abstract is enclosed herewith). Applicant need not teach what is well known to the art. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81, 94-95 (Fed. Cir. 1986).

Moreover, it is not the structure of an agent that identifies it for selection in Applicant's screen for an agent with a second property, it is the function of that agent that identifies it for selection, e.g., an agent that enhances the transduction of a viral gene therapy vector in mammalian cells. Thus, the class of agents to be screened is adequately described.

Further, the functional property of the agents to be identified is adequately described, i.e., the agent inhibits ENaC expression or activity in mammalian cells having increased ENaC expression or activity as a result of increased transcription of DNA encoding one or more of the ENaC subunits, wherein the increased ENaC activity in the mammalian cells is relative to corresponding cells with a wild-type cystic fibrosis transmembrane receptor (CFTR).

Therefore, the specification satisfies the written description requirement for agents employed in or identified by the claimed method.

With regard to the genus of mammalian cells with increased ENaC activity or expression, the Examiner asserts that neither the specification nor the art teaches objective, quantitative values to determine whether the expression or activity of the ENaC subunits is increased in the enormous genus of mammalian cells and that a representative number of species has not been described by their complete structure. In support of this position, the Examiner points to Audige

et al. (Clin. Sci., 104:389 (2003)) and Bubien et al.(J. Biol. Chem., 276:8557 (2001)) to show that ENaC RNA levels fluctuate and that quantitative values of RNA and protein for ENaC subunits are not readily obtainable.

The Examiner is respectfully reminded that claim 2 recites that the selected agent is contacted *in vitro* with mammalian cells having increased expression or activity of ENaC having α, β and γ subunits of ENaC as a result of increased transcription of DNA encoding one or more of the subunits with an amount of the one or more agents effective to enhance transduction of a viral gene therapy vector, wherein the increased ENaC activity in the mammalian cells is relative to corresponding cells with a wild-type cystic fibrosis transmembrane receptor (CFTR).

Audige et al. determined the relative amount of ENaC subunit RNA and protein in rat kidneys after a single administration of puromycin aminonucleoside in saline, which induces nephrotic syndrome in the rats. Control rats were administered saline. Audige et al. report that ENaC mRNA increased on days 1 and 2 after puromycin aminonucleoside administration but decreased by day 3, and that ENaC subunit proteins did not increase during puromycin aminonucleoside-induced sodium retention.

Bubien et al. concluded, based on four independent methodologies including analyses of protein and electrophysiological findings, that the mutation responsible for Liddle's disease induces excessive ENaC expression in human lymphocytes relative to controls. It is disclosed that the control lymphocytes were from normal individuals.

Audige et al. and Bubien et al. evidence that ENaC RNA or protein levels are detectable, and that increased levels of that RNA or protein can be detected, in mammalian cells, e.g., rat kidneys and human lymphocytes. Therefore, relative values for ENaC RNA and protein can be readily determined in different types of mammalian cells.

The Examiner also alleges that the claims and specification do not establish a nexus between ENaC expression and wild-type CFTR (page 7 of the Office Action) yet asserts that those of ordinary skill in the art have recognized a reciprocal expression relationship between ENaC and CFTR (page 21 of the Office Action). Clarification on this apparent contradiction is respectfully requested.

At page 11 of the Office Action, the Examiner asserts that claim 2 recites "aberrant". The Examiner is requested to consider that the last instance of the word "aberrant" in claim 2 was deleted in the Amendment filed on May 14, 2008.

As the specification and claims are in compliance with the "written description" requirement of § 112(1), withdrawal of the § 112(1) "written description" rejection is respectfully requested.

With regard to enablement, the Examiner asserts that 1) the specification does not define "mammal"; 2) the specification fails to disclose the necessary guidance to select an agent that enhances the transduction of a viral gene therapy vector; 3) the specification fails to disclose a nexus between the structure of a compound and its functional properties; 4) the specification fails to disclose the ability to predictably identify the enormous genus of structurally diverse agents possessing the functional properties; and 5) given the absence of definitions, disclosure and guidance in the specification, the artisan must perform extensive screening of an enormous genus of compounds to identify an agent with the desired property and that, unlike the monoclonal antibodies in Hybritech, which share a common structural and functional property, the list of agents disclosed in the present application do not share a common structural and functional property.

It is well-settled that it is not necessary that a patent applicant have prepared and tested all the embodiments of his invention in order to meet the requirements of § 112. In re Angstadt, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976). Furthermore, enablement is not precluded by the necessity for some experimentation, such as routine screening. The key word is "undue" not "experimentation." In re Angstadt, 190 U.S.P.Q. 214, 219 (C.C.P.A. 1976). In fact, a considerable amount of experimentation is permissible if it is merely routine, or the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should take. Ex parte Jackson, 217 U.S.P.Q. 804, 807 (Bd. App. 1982). Thus, if Applicant's invention is disclosed so that one of ordinary skill in the art can practice the claimed invention, even if the practice of the invention by the art worker includes routine screening or some experimentation, Applicant has complied with the requirements of 35 U.S.C.

§ 112, first paragraph. In re Angstadt, 190 U.S.P.Q. 214 (C.C.P.A. 1976); Ex parte Jackson, 217 U.S.P.Q. 804 (Bd. App. 1982).

With respect to "mammal" and agents that enhance viral transduction, the Examiner is respectfully reminded that Applicant need not teach what is well known to the art. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81, 94-95 (Fed. Cir. 1986). Any mammalian cell with increased ENaC expression or activity may be employed in the method of the invention. As shown in Audige et al. and Bubien et al., it was within the skill of the art at the time of Applicant's filing to identify those cells. And as discussed above, agents that enhance viral transduction were known to the art prior to Applicant's filing. Further, Applicant's specification discloses methods to detect the enhancement of viral transduction by an agent.

The disclosed nexus between doxorubicin and other agents to be screened in the method of the invention is that they are selected from agents that enhance viral transduction.

35 U.S.C. § 112, first paragraph, requires that the specification provide an enabling disclosure for the claimed invention. The claims are directed to a screening method and the agents to be screened have a certain functional property and the agents identified by the screen have a different functional property. The fact that the property of the agents to be screened in the assay, and the property to be identified, are not necessarily related to a particular chemical structure is irrelevant to whether the specification has enabled the claimed invention.

The fact that the outcome of the claimed screening program may be unpredictable is precisely why a screening program is carried out. It simply cannot reasonably be contended that a program to locate biomolecules with target biological or physical properties would not be carried out by the art because the results cannot be predicted in advance.

The Federal Circuit has explicitly recognized that the need, and methodologies required, to carry out extensive synthesis and screening programs to locate biomolecules with particular properties do not constitute undue experimentation. In re Wands, 8 U.S.P.Q.2d 1400, 1406-1407 (Fed. Cir. 1988), the Court stated:

> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.

Likewise, practitioners in the art related to the present application would be well-equipped to screen agents to identify those with the recited dual activities. See also, Hybritech Inc. v. Monoclonal Antibodies Inc., 231 U.S.P.Q. 81, 84 (Fed. Cir. 1986) (evidence that screening methods used to identify characteristics [of monoclonal antibodies] were available to art convincing of enablement). Thus, the fact that a given claim may encompass a variety of agents is not dispositive of the enablement issue, particularly in an art area in which the level of skill is very high and in which screening of large numbers of compounds has been standard practice for at least ten years (Ex parte Forman, 230 U.S.P.Q.2d 456 (Bd. App. 1986).

Evidence that screening numerous compounds to detect the effect of the compound is within the skill of the art, is provided in the abstracts for Cheng et al. and Dhanak et al.

It is Applicant's specification that provides the requisite predictability that agents that enhance viral transduction may also inhibit increased ENaC activity or expression.

Contrary to the Examiner's assertion, the structure of monoclonal antibodies secreted from a set of hybridomas obtained from fusions with cells exposed to the same immunogen, is quite likely to be different, e.g., the different clones will secrete antibodies with different primary amino acid sequences including different Ig heavy and light chain classes. Those monoclonal antibodies may also recognize different nonoverlapping epitopes on the immunogen, have different affinities and avidities for the immunogen, and different utilities, e.g., in immunoprecipitation or in ELISAs. That is, monoclonal antibodies obtained from the same fusion may have different functions. Moreover, a screen for antibodies with desired properties generally includes a functional screen, i.e., binding to an immunogen. Binding of two different monoclonal antibodies to the identical immunogen (a functional property) does not require that the two antibodies have the same or even substantially the same structure.

Therefore, the specification, in view of the knowledge in the art, clearly satisfies the enablement requirement of § 112(1). Thus, withdrawal of the enablement rejection under § 112(1) is respectfully requested.

The 35 U.S.C. § 103 Rejections

Claims 1, 4-5, 13, 15-16, 20, and 23 were rejected under 35 U.S.C. § 103(a) as being unpatentable ovcr Duan et al. (J. Clin. Invest., 105:1573 (2000)) in view of Kiyomiya et al. (Cancer Res., 61:2567 (2001). Claims 2, 4, 6-7, 13, 15-16, 20, and 23 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Duan et al. in view of Kiyomiya et al. and Maitra et al. (Am. J Physiol. Cell Physiol., 280:C1031 (2001)). These rejections are respectfully traversed.

The rejection of claims 1, 4-5, 13, 15-16, 20, and 23 under § 103(a) over Duan et al. in view of Kiyomiya et al. is obviated in view of the cancellation of claim 1.

Duan et al. disclose that LLnL or Z-LLL enhances AAV transduction in epithelial cells *in vitro* and *in vivo* and in liver *in vivo*.

The Examiner asserts that Duan et al. teach a screening method to identify one or more agents with dual activities. Although LLnL is a proteasome modulator and was found to enhance AAV transduction, Duan et al. do not disclose a screening assay where agents that enhance viral transduction are screened for inhibition of ENaC activity or expression.

Kiyomiyo et al. disclose a mechanism for the nuclear transport of adriamycin (doxorubicin), which may involve binding of adriamycin to the 20S proteosome. There is nothing in Kiyomiyo et al. related to virus transduction or ENaC.

Maitra et al. relate that a single dose of doxorubicin increased total cellular CFTR protein expression, surface CFTR protein expression and CFTR-associated chloride secretion in T84 epithelial cells, and increased mutant CFTR cell surface expression and chloride secretion in stably transfected MDCK cells. There is nothing in Maitra et al. related to virus transduction or ENaC.

Duan et al., Kiyomiya et al. or Maitra et al., individually or in combination with each other, do not disclose or suggest contacting mammalian cells having increased expression or activity of ENaC with an agent that enhances viral gene therapy vector transduction and identifying one or more agents which also inhibit expression or activity of ENaC.

The Examiner asserts that it would have been obvious to one of ordinary skill in the art to substitute the proteasome inhibitor agent LLnL as taught by Duan et al. with the proteasome inhibitor agent doxorubicin as taught by Kiyomiya et al. in a screening method to identify one or

more agents with dual activities with a reasonable expectation of success because the simple substitution of one known element for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention.

The Examiner has not set forth a proper *prima facie* case of obviousness because the cited portions of Duan et al. and Kiyomiya et al., individually or in combination with each other, and the reasoning given above, do not provide the recited subject matter. For instance, Applicant is unable to find in Duan et al. and Kiyomiya et al., among other things, screening an agent that enhances viral transduction for inhibition of ENaC activity or expression. Applicant is unable to find in the Office Action a proper reason that remedies this deficiency.

Assuming, for the sake of argument, that one of skill in the art may have been motivated to detect whether agents other than LLnL or Z-LLL enhanced AAV transduction, there is nothing in the cited art that provides a reasonable expectation that proteosome inhibitors other than LLnL or Z-LLL would alter AAV transduction. It is only Applicant's specification that provides the predictability that proteosome modulators as a class may alter AAV transduction.

The Examiner alleges that doxorubicin was recognized as a DNA damaging agent and DNA damaging agents were recognized in the art to possess AAV transduction enhancement properties (citing Russell et al. (Proc. Natl. Acad. Sci. USA, 92:5719 (1995)). However, not all of the agents disclosed in Russell et al. appear to be "DNA damaging agents" nor enhanced AAV transduction. Russell et al. disclose that aphidicolin or hydroxyurea (both inhibitors of DNA synthesis), hydroxyurea (an inhibitor of ribonucleotide reductase), and some topoisomerase inhibitors, i.e., etoposide and camptothecin, but not others, i.e., novobiocin and amsacrine, increased rAAV transduction of human fibroblasts (page 5720). It is also disclosed that inhibitors of RNA or protein synthesis, i.e., actinomycin D and cycloheximide, did not increase rAAV transduction (page 5720).

The Examiner asserts that an artisan would be motivated to substitute the human bronchial airway epithelial cells of Duan et al. with mammalian cells having aberrant ENaC expression, where the mammalian cells do not express functional CFTR, as taught by Maitra et al., because Maitra et al. teach that enhancing ΔF508-CFTR cell surface expression may be clinically useful in treatment of CF patients and that doxorubicin significantly increases

functional cell surface expression and activity of CFTR and ΔF508-CFTR. The Examiner continues asserting that the substitution would have a reasonable expectation of success because the simple substitution of one known element for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention.

The Examiner further asserts that it would have been obvious to modify the method of Duan et al. to comprise a step of assaying the mammalian cells that do not expression functional CFTR for decreased or inhibited ENaC expression or activity with a reasonable expectation of success because those of ordinary skill in the art have long-recognized that a reciprocal expression relationship between CFTR and ENaC, and decreased or inhibited ENaC expression or activity would be an indicator of increased expression of functional CFTR, which would be of therapeutic value for those patients in need.

The Examiner has not set forth a proper *prima facie* case of obviousness because the cited portions of Duan et al. and Maitra et al., individually or in combination with each other, and the reasoning given above, do not provide the recited subject matter. For instance, Applicant is unable to find in Duan et al., or Duan et al. and Maitra et al., among other things, screening an agent that enhances viral transduction for inhibition of ENaC activity or expression. Applicant is unable to find in the Office Action a proper reason that remedies this deficiency.

Moreover, none of the cited art provides a reasonable expectation that an agent that enhances viral transduction, e.g., AAV transduction, may also inhibit ENaC expression or activity.

Therefore, withdrawal of the § 103 rejections is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance, and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's representative at (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date November 13, 2008 By

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being filed using the USPTO's electronic filing system EFS-Web, and is addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on November 13, 2008.

CANDIS BUENDING

Name

Signature

Endosomal processing limits gene transfer to polarized airway epithelia by adeno-associated virus

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The restriction of viral receptors and coreceptors to the basolateral surface of airway epithelial cells has been blamed for the inefficient transfer of viral vectors to the apical surface of this tissue. We now report, however, that differentiated human airway epithelia internalize rAAV type-2 virus efficiently from their apical surfaces, despite the absence of known adeno-associated virus–2 (AAV-2) receptors or coreceptors at these sites. The dramatically lower transduction efficiency of rAAV infection from the apical surface of airway cells appears to result instead from differences in endosomal processing and nuclear trafficking of apically or basolaterally internalized virions. AAV capsid proteins are ubiquitinated after endocytosis, and gene transfer can be significantly enhanced by proteasome or ubiquitin ligase inhibitors. Tripeptide proteasome inhibitors increased persistent rAAV gene delivery from the apical surface >200-fold, to a level nearly equivalent to that achieved with basolateral infection. In vivo application of proteasome inhibitor in mouse lung augmented rAAV gene transfer from undetectable levels to a mean of 10.4 ± 1.6% of the epithelial cells in large bronchioles. Proteasome inhibitors also increased rAAV-2–mediated gene transfer to the liver tenfold, but they did not affect transduction of skeletal or cardiac muscle. These findings suggest that tissue-specific ubiquitination of viral capsid proteins interferes with rAAV-2 transduction and provides new approaches to circumvent this barrier for gene therapy of diseases such as cystic fibrosis.

J. Clin. Invest. 105:1573–1587 (2000).

Introduction

Cystic fibrosis (CF) is the most common inherited disease in the Caucasian population. Because the pulmonary manifestations are the most important pathological changes associated with morbidity and mortality in patients with CF, the initial phases of gene therapy for this disorder will target the lung airway epithelium. Several viral vectors, including adenovirus, lentivirus, retrovirus, and adeno-associated virus (AAV), have been used in strategies conceived for functional correction of the cystic fibrosis transmembrane conductance regulator (CFTR) gene defect in patients with CF. The development of AAV as a gene therapy vehicle for treating CF has several unique advantages based on its viral biology. For example, wild-type AAV infections are known to occur in the respiratory epithelium but have no known associated pathology. Additionally, recombinant adeno-associated virus (rAAV) can infect nondividing cells, and its genome can exist in either integrated or episomal forms.

Unfortunately, fully differentiated airway epithelia are extremely resistant to infection from the apical surface, not only with rAAV-2, but also with all other types of viral vectors currently in use (1, 2). Several potential rate-limiting steps may be responsible for the observed resistance of the apical airway surface to infection with these viruses. These include viral binding, endocytosis, endosomal processing, nuclear transport, uncoating, gene conversion, and transcription and translation of gene products. Several clues have assisted researchers in understanding the rate-limiting steps for recombinant viral transduction in the airway. In the case of adenovirus, partitioning of viral receptors and coreceptors to basolateral surfaces has clearly been shown, at least in part, to contribute to low infectivity from the apical surface of the airway (2). Similarly, it is thought that the absence of viral receptors at the apical surface may also significantly limit transduction by retroviruses (1). Recently, the heparin sulfate proteoglycan receptor (3), and FGFR-1 (4) and αVβ5 integrin (5) coreceptors for AAV-2, have been localized to the basolateral membrane of polarized airway epithelial cells (6, 7). This suggested that receptor abundance might also play a significant role in the low infectivity from the apical surface observed with rAAV-2. Studies on air-liquid interface cultures of primary human bronchial epithelia have demonstrated a 200-fold higher level of AAV-2 transduction from the basolateral, compared with apical membranes (6). Together with the fact that differentiated monolayers of airway cells are also resistant to AAV-2 infection (8), these studies suggest that aspects of either differentiation and/or polarity are responsible for reduced AAV infection from the apical surface.

1: J Gene Med. 2002 Mar-Apr;4(2):161-9.

High efficiency lentiviral gene delivery in non-dividing cells by deoxynucleoside treatment.

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BACKGROUND: Gene therapy has recently been advanced by the development of HIV-based vectors that are able to transduce some non-dividing cells. The manipulation of most non-dividing cells remains, however, scarcely efficient. One of the biological mechanisms postulated to prevent powerful transduction of quiescent cells by lentiviral vectors is the paucity of deoxynucleotides (dNTPs). In this study, a novel delivery strategy is developed to improve significantly the efficiency of HIV-based vectors in transducing non-dividing cells. This approach is based on increasing the intracellular availability of dNTPs by incubating target cells with the dNTP precursors, deoxynucleosides (dNSs). METHODS: Mature human monocyte-derived macrophages (14-21 days old) were transduced at a low multiplicity of infection (MOI) of HIV vectors carrying a reporter gene. dNSs were added to the medium during transduction (5 mM dNS) and immediately before post-transduction culture (2.5 mM dNS). Macrophages were harvested 2-7 days after transduction and assayed for transgene expression by cytofluorimetry. RESULTS: The addition of dNS to the medium significantly enhanced the efficiency of transduction of human macrophages by HIV-based vectors. The percentage of cells expressing the transgene rose up to 50% in the presence of dNS, increasing the basal transduction levels up to 35-fold (average=10.8-fold). Furthermore, treatment with dNTP precursors compensated for the wide inter-donor variability, allowing the highest enhancement effects in donors with the lowest basal transduction efficiencies. CONCLUSIONS: This is the first demonstration that a single treatment of non-dividing target cells with exogenous dNS can enhance the efficiency of lentiviral-mediated transduction of cells, allowing for high efficiency gene transfer. The effects of dNTP precursors compensated for both the poor basal levels and the wide inter-donor variability, two major limitations for the transduction of non-dividing cells. Macrophages are a representative model of cells whose permissiveness to gene delivery was increased up to levels suitable for genetic manipulation applications. This simple approach might be transferred to a broader range of quiescent cell types that are scarcely susceptible to lentiviral-based gene delivery due to low dNTP levels. Copyright 2002 John Wiley & Sons, Ltd.

PMID: 11933217 [PubMed - indexed for MEDLINE]

1: Cancer Res. 2003 Jul 15;63(14):4128-35.

CpG oligodeoxynucleotides enhance the capacity of adenovirus-mediated CD154 gene transfer to generate effective B-cell lymphoma vaccines.

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Activation of CD40 by CD154 induces antigen-presenting cells (APC) to express immune costimulatory molecules, thereby enhancing their APC activity. Oligonucleotides (ODN), containing immunostimulatory DNA sequences (ISS) with nonmethylated CpG dinucleotides in a defined motif, also can induce similar changes in APC. In this study, we examined whether infection with recombinant adenovirus (Ad) encoding CD154 and/or treatment with ISS-ODN could enhance the capacity of A20 murine B lymphoma cells to function as APCs capable of inducing a syngeneic antilymphoma immune response. High-level expression of CD154 after infection with Ad-CD154 induced up-regulation of immune costimulatory molecules on A20 cells, as did incubation with ISS-ODN. Treatment of A20 cells with ISS-ODN also enhanced surface expression of alphav integrins, making them significantly more susceptible to Ad infection than nontreated A20 cells. In syngeneic mixed-lymphocyte reactions with BALB/c splenocytes, A20 cells activated with ISS-ODN and then transduced with Ad-CD154 were significantly more effective APCs than Ad-CD154 transduced cells, which, in turn, were significantly more effective than A20 cells treated with ISS-ODN alone. Also, injection of mice with ISS-activated, Ad-CD154-infected cells induced significantly better A20-specific immune responses against A20 cells, as assessed via enzyme-linked immunospot analysis in vitro and immune prophylaxis against subsequent challenge with A20 lymphoma cells in vivo. These data demonstrate that CpG-containing oligonucleotides can serve as an adjuvant for Ad-mediated gene therapy of B-cell malignancies.

PMID: 12874017 [PubMed - indexed for MEDLINE]

1: Gene Ther. 1998 Sep;5(9):1180-6.

Links

Enhanced in vitro and in vivo gene delivery using cationic agent complexed retrovirus vectors.

Themis M, Forbes SJ, Chan L, Cooper RG, Etheridge CJ, Miller AD, Hodgson HJ, Coutelle C.

Division of Biomedical Sciences, Imperial College School of Medicine, London, UK.

Retroviruses are, at present, the most efficient integrative vectors available for gene delivery. However, these viruses are still limited by relatively low titres. Although several protocols exist to improve virus titre most of them are time-consuming and unable to provide sufficient virus for in vivo applications. Virus titre can be enhanced by polybrene and other cationic agents. By investigating a broad range of cationic agents for their ability to enhance virus infectivity we found that both ecotropic and amphotropic retrovirus infection could be increased. In particular, the lipopolyamine dioctadecylamidoglycylspermine (DOGS) gave up to one order of magnitude enhancement above polybrene-mediated infection without cytotoxicity. To increase virus infectivity further we combined the enhancing effect of DOGS on virus infectivity with concentration of virus particles by ultrafiltration to reach titres of 1 x 10(9) IU/ml. The in vivo transduction of regenerating rat liver, by an amphotropic retrovirus was increased approximately five-fold by the addition of DOGS compared with virus alone. There was no animal toxicity observed following the administration of DOGS. The improved transduction efficiency seen both in vitro and in vivo following the co-administration of DOGS/virus complexes may be useful for future gene therapy applications.

PMID: 9930318 [PubMed - indexed for MEDLINE]

Related Articles

- Cationic liposomes enhance the rate of transduction by a recombinant retroviral vector in vitro and in vivo. [J Virol. 1998]
- Ultrasound enhances retrovirus-mediated gene transfer. [Prep Biochem Biotechnol. 2007]
- Enhanced transduction efficiency of retroviral vectors coprecipitated with calcium phosphate. [illegible]
- [illegible] cationic liposomes enhance retroviral transduction. [Nat Biotechnol. 1996]
- Rapid concentration and purification of retrovirus by flocculation with Polybrene. [J Biotechnol. 2006]

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1: J Biol Chem. 2002 Oct 11;277(41):38322-7. Epub 2002 Aug 6.

Identification and biological characterization of heterocyclic inhibitors of the hepatitis C virus RNA-dependent RNA polymerase.

Dhanak D, Duffy KJ, Johnston VK, Lin-Goerke J, Darcy M, Shaw AN, Gu B, Silverman C, Gates AT, Nonnemacher MR, Earnshaw DL, Casper DJ, Kaura A, Baker A, Greenwood C, Gutshall LL, Maley D, DelVecchio A, Macarron R, Hofmann GA, Alnoah Z, Cheng HY, Chan G, Khandekar S, Keenan RM, Sarisky RT.

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The hepatitis C virus (HCV) NS5B protein encodes an RNA-dependent RNA polymerase (RdRp), the primary catalytic enzyme of the HCV replicase complex. We established a biochemical RNA synthesis assay, using purified recombinant NS5B lacking the C-terminal 21 amino acid residues, to identify potential polymerase inhibitors from a high throughput screen of the GlaxoSmithKline proprietary compound collection. The benzo-1,2,4-thiadiazine compound 1 was found to be a potent, highly specific inhibitor of NS5B. This agent interacts directly with the viral polymerase and inhibits RNA synthesis in a manner noncompetitive with respect to GTP. Furthermore, in the absence of an in vitro-reconstituted HCV replicase assay employing viral and host proteins, the ability of compound 1 to inhibit NS5B-directed viral RNA replication was determined using the Huh7 cell-based HCV replicon system. Compound 1 reduced viral RNA in replicon cells with an IC(50) of approximately 0.5 microm, suggesting that the inhibitor was able to access the perinuclear membrane and inhibit the polymerase activity in the context of a replicase complex. Preliminary structure-activity studies on compound 1 led to the identification of a modified inhibitor, compound 4, showing an improvement in both biochemical and cell-based potency. Lastly, data are presented suggesting that these compounds interfere with the formation of negative and positive strand progeny RNA by a similar mode of action. Investigations are ongoing to assess the potential utility of such agents in the treatment of chronic HCV disease.

PMID: 12167642 [PubMed - indexed for MEDLINE]